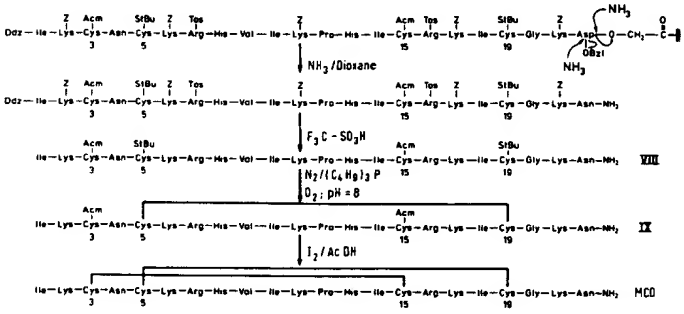
I and II reacted on the carrier to form V (53% based on II-gel polymer) and III and IV reacted on the carrier to give VI (24% based on IV-gel polymer). V was again released from the carrier, purified, and combined with VI on the carrier to give the fully protected final product VII (32% based on VI-gel polymer). The scheme of fragment syntheses and the formation of the fully protected sequence VIII has already been briefly described[7].



Scheme 2. Liberation of the MCD peptide from carrier-bound final product VII.

The product VII of the Merrifield synthesis was released from the carrier as the diamide by treatment with ammonia-saturated dioxane (Scheme 2). The release of VII from polystyrene gel was practically complete (98.5%) and after chromatography on Sephadex LH 20/DMF resulted in completely protected MCD peptide (MW 4067). All the benzyloxycarbonyl and toluenesulfonyl groups were removed with trifluoromethanesulfonic acid, the sulfur-protecting groups being left intact in positions 3, 5, 15, and 19. No aromatic protons were detected in the ^{1}H-NMR spectrum, while the signals of the sulfur-protecting groups remained unchanged.

After ion exchange and gel chromatography, 507 mg of Cys(Acm)^{3,15},Cys(StBu)^{5,19}-MCD peptide (VIII) was obtained (71% based on the amount of VII released from the carrier).

Reduction with tributylphosphane[8] selectively removed the StBu protecting groups in positions 5 and 19, and the disulfide bridge 5—19 was formed by air oxidation at pH 8; there resulted 276 mg of Cys(Acm)^{3,15}-MCD peptide (IX) (61% based on VIII).

In order to bring about selective linkage of the 3—15 disulfide bridge, the Acm protecting groups were removed from 126 mg of IX by iodine in acetic acid solution[9]. Oxidation was halted with ascorbic acid and the final product immediately chromatographed on Sephadex G25 with 0.1 N acetic acid. The fraction containing the most synthetic MCD peptide initially gave 16 mg of pure product after purification. It was shown by electrophoresis (paper, polyacrylamide normal and SDS gel) and chromatography (HPLC, Zorbax ODS; acetonitrile/water 35/65, isocratic) to be identical with the natural product. Amino acid analysis (calculated values given in parentheses): Cys 4.2(4); Ile 4.0(4); Lys 5.0(5); Arg 2.3(2); His 1.8(2); Val 0.8(1); Pro 1.4(1); Gly 1.5(1); Asp 2.4(2).

In the CD spectrum, the synthetic MCD peptide shows the same four bands between 190 and 240 nm as the natural product; however, the spectra are not congruent. This suggests gradual racemization of particularly endangered sites (histidine, cysteine). The degranulating effect of the synthetic product was tested on rat mast cells. The synthetic MCD peptide exhibited 35% of the histamine-releasing activity of the natural product.

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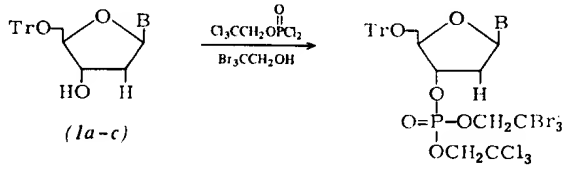
Selective Electrochemical Removal of Protecting Groups in Nucleotide Synthesis

By *Joachim Engels*[*]

In the synthesis of nucleotides by the triester method[1] a completely protected nucleoside-3′-phosphate in the triester form *(2)* can be used as synthon for construction of the oligonucleotide chain. A major problem in the synthesis is the selective cleavage of an ester moiety. We report here on the selective deprotection of such synthons by controlled potential electroreduction[2]. Two important advantages make this approach attractive: Firstly, the 2,2,2-trichloro- and -bromoalkyl esters of phosphoric acid are quite stable towards both basic and acidic hydrolysis[3], so that a wide range of acid- and alkali-labile protecting groups can be used on the sugar and on the base. Secondly, owing to the reaction mechanism[4], the danger of an isomerization during cleavage of an ester function is far less than during attack of the reagent at phosphorus.

The protected nucleosides *(1a—c)* were allowed to react with 2,2,2-trichloroethylphosphoric dichloride[5] and then with 2,2,2-tribromoethanol. The triesters *(2a—c)* can be isolated in 85—90% yield by chromatography on silica gel.



(1a-c) *(2a-c)*

(a), B = Thyminyl
(b), B = N^4-Benzoylcytosyl
(c), B = N^6-Benzoyladenyl

Tr = Trityl or monomethoxytrityl

The detritylation of *(2a—c)* was achieved with 1% trifluoroacetic acid in CH_2Cl_2 or with BF_3Et_2O/MeOH in CH_2Cl_2[6]: the resulting crystalline esters *(4a—c)* are stable and can be lengthened in the 5′-direction. It is possible to cleave the tribromoethyl protecting group quantitatively from *(2a—c)* by controlled potential electroreduction[7] at a mercury cathode at −0.5 to −0.6 V. The reaction is carried out in acetonitrile/pyridine with $LiClO_4$ as supporting electrolyte: the diesters *(3a—c)* which are formed can be used for the condensation reaction directly after extraction with $CHCl_3$. Reaction of TPS with the triesters *(4a—c)* afforded *(6)*. The reactions proceed very smoothly. The nine possible combinations of

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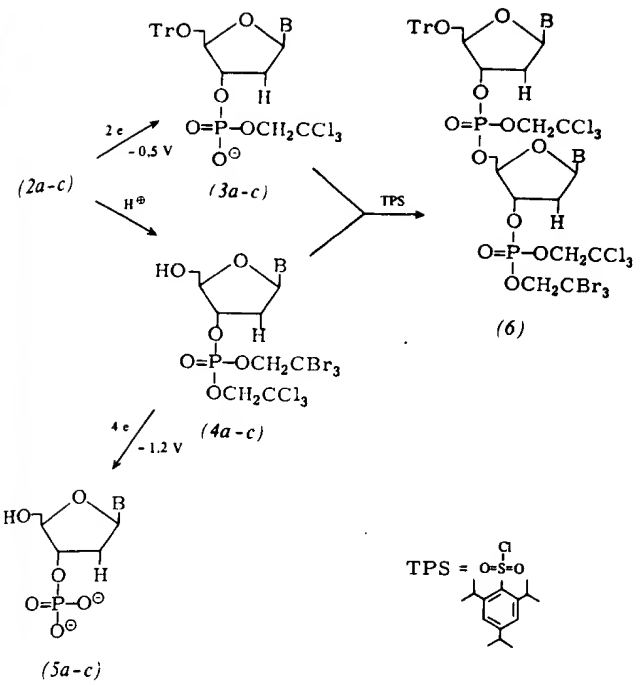

(6) can then be cleaved by selective electroreduction and lengthened in the 3'-direction or be used for block condensation after acid cleavage at the 5'-terminal. Complete electrochemical deprotection to give the free phosphates was carried out on the diesters *(4a—c)*. On reduction in dimethylformamide (DMF) with tetrabutylammonium tetrafluoroborate as supporting electrolyte, *(4a)* and *(4c)* can be smoothly reduced at −1.2 to −1.4 V to *(5a)* and *(5c)* respectively in 90% yield. The dichloroethyl ester, *i.e.* the byproduct cited in [4], is formed to the extent of 2% at most and is virtually inseparable from the trichloroethyl ester (reactant).

Experimental

Reduction of *(2a—c)*: The reduction is carried out at room temperature under nitrogen in a partitioned electrolytic cell[8] (Pt-anode, Hg-cathode, Ag-wire; Nafion 125 membrane) using a 0.5 M solution of $LiClO_4$ in pyridine/CH_3CN (1:5) and 0.5—1 mmol *(2a—c)* as catholyte and 0.5 M $LiClO_4$ and 1—2 mmol pyridine as anolyte. The cathode potential is potentiostatically adjusted to −0.5 V (Ag-wire) and the uptake of electrons monitored coulometrically. When the current has reached its residual value the reaction is complete. After removal of solvent, partition of the residue between water and chloroform, extraction with chloroform, and drying of the chloroform phase with Na_2SO_4 followed by filtration and removal of solvent, *(3a—c)* is obtained and can be used directly for further reaction in pyridine.

Reduction of *(4a—c)*: The reduction is carried out at room temperature under nitrogen in the same type of cell as described above using 0.5 M tetrabutylammonium tetrafluoroborate in DMF/CH_3CN (1:1) and 0.5—1 mmol *(4a—c)* as catholyte and the same anolytes as above plus an additional 1—2 mmol 2,6-lutidine. The cathode potential is adjusted to −1.2 V (Ag-wire). On completion of reaction, extraction of the supporting electrolyte with chloroform and chromatography of the aqueous phase on RP8 or RP18 silica gel with water or water/ethanol as eluent affords *(5a—c)*.

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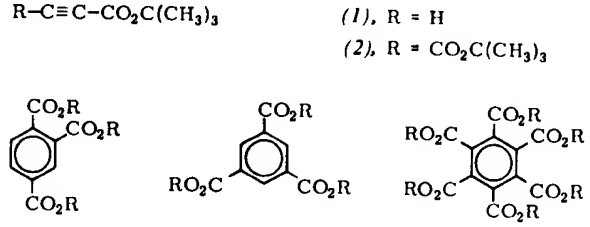
Di-*tert*-Butyl Acetylenedicarboxylate and Its Cyclotrimerization

By *Wolfgang Sucrow* and *Fritz Lübbe*[*]

For the synthesis of ene-hydrazines[1] we required as starting material di-*tert*-butyl acetylenedicarboxylate *(2)*, which, surprisingly, had previously not been described in the literature. Following the procedure used for the preparation of tert-*butyl* propiolate *(1)*[2] we have now been able to obtain *(2)* as a crystalline product, m.p. 35—36°C [^{1}H-NMR (90 MHz, $CDCl_3$): singlet, δ = 1.50] from acetylenedicarboxylic acid and isobutene.



(a), R = $C(CH_3)_3$; *(b)*, R = H

In spite of the bulky *tert*-butyl residue the esters *(1)* and *(2)* can be trimerized with dicarbonylbis(triphenylphosphane)nickel(0)[3]. *(1)* furnishes tri-*tert*-butyl trimellitate *(3a)* [oil, b.p. 165—170°C (bath)/0.03 torr, Kugelrohr] and tri-*tert*-butyl trimesitate *(4a)* [from petroleum ether, m.p. 218°C (dec)] in the ratio 6:1, while, according to the NMR spectrum, the known trimethyl esters are formed in the ratio 12:1. The total yield of *(3a)* and *(4a)* after chromatographic separation is 55%.—Under somewhat more severe conditions, *(2)* trimerizes to hexa-*tert*-butyl mellitate *(5a)* in 44% yield [^{1}H-NMR (90 MHz, $CDCl_3$): singlet, δ = 1.59]. The crystals obtained from petroleum ether/chloroform decompose without melting above 180°C into mellitic acid *(5b)* and isobutene. In the case of the ester *(4a)* complete decomposition to *(4b)* takes place following previous melting.

In contrast to *(1)*, compounds *(2)* and *(3a)* also decompose to acetylenedicarboxylic acid and *(3b)*, respectively, on attempted distillation above 165°C and 204°C under normal pressure.—The new compounds *(2)*, *(3a)*, *(4a)*, and *(5a)* gave correct elemental analyses.

As shown by the cyclotrimerization, the *tert*-butyl groups in *(2)* do not give rise to much steric hindrance. The numerous reactions realizable with dimethyl acetylenedicarboxylate can thus presumably also be carried out with the di-*tert*-butyl ester *(2)*. This offers advantages, *e.g.* easy cleavage of the ester without use of bases.

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